Autotrophic Picoplankton in a Regulated Coastal River in New South Wales

TSUYOSHI KOBAYASHI[1], SIMON WILLIAMS[2] AND AMANDA KOTLASH[3]

[1]AWT Environment Science and Technology, PO Box 73, West Ryde NSW 2114 and Centre for Marine and Coastal Studies, University of New South Wales, Sydney NSW 2052 (present address: NSW Environment Protection Authority, PO Box A290, Sydney South NSW 1232);
[2] AWT Environment Science and Technology, PO Box 73, West Ryde NSW 2114 (present address: Sydney South Coast Region, Department of Land and Water Conservation, PO Box 867, Wollongong NSW 2520); and
[3]AWT Environment Science and Technology, PO Box 73, West Ryde NSW 2114 (present address: Blue Mountain City Council, PO Box 189, Katoomba NSW 2380)

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Cell density, cell type and vertical distribution of autotrophic picoplankton (APP, cell size 0.2-2 μm) were examined for a year from September 1992 at three freshwater sites in the Hawkesbury-Nepean River. During the study, mean cell density of APP at 1 m deep varied seasonally two orders of magnitude from 2.2×10^3 to 3.2×10^5 cells mL^{-1}. At upstream sites of Penrith and North Richmond, higher cell density was observed from summer to autumn. There were three cell types of APP (i.e. coccoid, ellipsoid and rod-shaped). Proportionally, coccoid cells increased downstream from 25 to 52 % of total cells, whereas ellipsoid and rod-shaped cells decreased downstream from 64 to 47 % and from 11 to 1 % of total cells, respectively. The vertical distribution of APP (1 and 4 m deep), examined for 9 months at North Richmond, showed that overall mean density at 1 m was significantly higher than overall mean density at 4 m. Overall, the cell density of APP at 1 m deep was positively correlated with temperature and total chlorophyll *a*. The present results suggest that APP may need to be incorporated into a conceptual model of river plankton food webs.

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KEYWORDS: environmental factors, Hawkesbury-Nepean River, phycoerythrin-rich picocyanobacteria, plankton food webs.

INTRODUCTION

In fresh waters, the presence of autotrophic picoplankton (APP, cell size: 0.2-2.0 μm, Sieburth et al. 1978) has been reported from lakes of various trophic states in the Northern and Southern Hemispheres (Paerl 1977; Stockner and Antia 1986; Burns and Stockner 1991; Weisse and Kenter 1991; Jasser 1997; Vörös et al. 1998). The ubiquitous and often abundant presence ($>10^5$ cells ml^{-1}) of APP in lakes has prompted many ecological studies to investigate the relationships between the population dynamics of APP and physico-chemical and biological conditions (see Weisse 1993 for review). Some studied also have focused on the trophic role of APP in aquatic food webs, especially in the context of

other microzooplankton (Stockner 1991; Weisse 1993).

In terms of the pattern of associations between environmental factors and APP in lakes, the abundance of APP may positively correlate with temperature (Pick and Carron 1987; Kennaway and Edwards 1989) but negatively correlate with intensity of zooplankton grazing (Weisse 1988; Fahnenstiel et al. 1991). The relationship between APP abundance and lake productivity may vary according to the trophic states (i.e. positive relationship in oligotrophic lakes and negative relationship in meso- and eutrophic lakes) (Stockner and Shortreed 1991; Burns and Stockner 1991). Thermal stratification may affect the vertical distribution of APP, largely confining their abundant presence above the thermocline (Fahnenstiel et al. 1991). In addition, these environmental factors may produce complex interaction effects on the temporal variation in the composition and abundance of APP (Rhew et al. 1999).

Despite detailed ecological studies of lake APP, such studies are few for river APP. In the present study, basic ecological aspects of APP were examined for a year in the freshwater portion of the Hawkesbury-Nepean River, a regulated coastal river in New South Wales. The present study aimed to examine 1) the seasonal and horizontal (longitudinal) variation in cell density and cell type of APP, 2) the vertical distribution of APP, and 3) the pattern of seasonal associations between river environmental variables and cell densities of APP.

MATERIALS AND METHODS

Study Sites

The Hawkesbury-Nepean River flows through the Illawara range to its mouth north of Sydney; the river has a catchment area of 32000 km^2 and a main channel length of about 320 km. The river flow has been regulated by five major dams on its headwaters and partly by more than 13 weirs. The present study was conducted at three freshwater sites. They were Penrith (non-tidal, about 180 m wide and 2 m deep), North Richmond (tidal limit, 120 m wide and 6 m deep) and Sackville (tidal, 200 m wide and 6 m deep) (see Fig. 1 in Kobayashi et al. 1998 for locations of sites).

Sampling, Enumeration and Cell Type Measurement

At each site, four replicates of water sample (100 ml each) were collected by using a Haney-type trap (Gawler and Chappuis 1987) from a depth of 1 m between 10:00 and 14:00. At North Richmond, additional samples were collected from a depth of 4 m between September 1992 and March 1993 to investigate the vertical distribution of APP (i.e. between 1 m and 4 m deep). All samples were immediately fixed with a 2% filtered (0.2 μm pore size) buffered-formaldehyde solution (buffer: sodium tetraborate), and were transported to the laboratory with ice and stored at 4°C in the dark.

In the laboratory, samples were initially filtered through a 3 μm polycarbonate filter (25 mm in diameter; Millipore) to remove larger phytoplankton and zooplankton (Hawkey and Whitton 1991). Subsamples (3-10 ml) of these were then drawn onto a 0.2 μm black polycarbonate filter (25 mm in diameter) under low vacuum pressure (<150 millibars). A cellulose acetate filter (pore size 0.45 μm) was placed between the black polycarbonate filter and the filter holder as a backing to obtain an even vacuum (Hawkey and Whitton 1991). The black polycarbonate filter was then mounted on a glass slide, with a drop of immersion oil placed on top of the filter before gently affixing a cover slip. The APP cells (* 2 μm in any dimension) were counted using fluorescence microscopy at a magnification of x1000, on a Nikon Diaphot-TMD inverted microscope, equipped with the standard G-2A green excitation filter set (excitation filter EX510-560, dichroic mirror DM580 and barrier filter BA590) and a 100-W mercury lamp. The cells that fluoresced red were counted. These cells were assumed to be phycoerythrin-rich picocyanobacteria (MacIsaac and Stockner 1993). Thus, strictly speaking, the present study is most likely to have estimated a portion of the entire APP assemblage that may include eukaryotic cells. A minimum of 200 cells or all cells that appeared in a maximum of 40 fields of view were counted for

Table 1. Summary statistics for environmental variables in the Hawkesbury-Nepean River between September 1992 and August 1993. Mean and range in parentheses are shown. n = the number of observations

Site	Width and depth* (m)	Temperature (°C)	Total phosphorus ($\mu g\ l^{-1}$)	Total nitrogen ($mg\ l^{-1}$)	Chlorophyll *a* ($\mu g\ l^{-1}$)
Penrith	180 and 2	20.6 (8.0-27.5), n=23	12.8 (5.0-74.0), n=22	0.47 (0.26-0.89), n=23	2.0 (0.3-4.2), n=23
North Richmond	120 and 6	19.3 (11.0-28.9), n=24	34.3 (21.0-59.0), n=22	0.97 (0.38-1.56), n=24	11.9 (1.8-22.9), n=21
Sackville	200 and 6	19.3 (12.9-27.9), n=23	40.5 (15.0-185.0), n=22	1.83 (0.84-3.20), n=22	23.7 (4.5-55.5), n=22

*Approximate width and depth of river channel. Width was read from topographic maps (scale: 1:25000).

expressed as the number of cells ml^{-1}.

Between December 1992 and August 1993, the proportional occurrences of morphological cell types of APP were investigated from 50 cells randomly selected from the pooled sample on each sampling date at each site.

River Environmental Variables

Temperature (°C) was measured with a Yeo-Kal Model 603 oxygen/temperature meter at 0.5 m depth. Water samples were collected for analysis of total phosphorus ($\mu g\ l^{-1}$), total nitrogen ($mg\ l^{-1}$) and total chlorophyll a ($\mu g\ l^{-1}$) in a Niskin-type bottle at 0.5 m depth. The samples were analysed by the methods described in Clesceri et al. (1989). Data on flow rate ($10^6\ l\ day^{-1}$) over Penrith weir were provided by AWT Environmental Science and Technology Division.

Statistical Analysis

A simple correlation analysis was used to detect any significant association between river environmental variables and APP cell densities at each site (α=0.05). All data except temperature were transformed by $\log_{10}$ to meet the assumptions of normality and homoscedasticity. All data analyses were made using the SAS computer programs (Anon. 1989).

RESULTS

River Environmental Conditions

Between December 1992 and November 1993, flow rate over Penrith weir ranged from 2.3×10^7 to $4.1 \times 10^9\ l\ day^{-1}$ (Fig. 1). Overall, temperature was in the range 8-28.9 °C. The means and ranges of concentrations of total phosphorus, total nitrogen and total chlorophyll *a* increased downstream (Table 1).

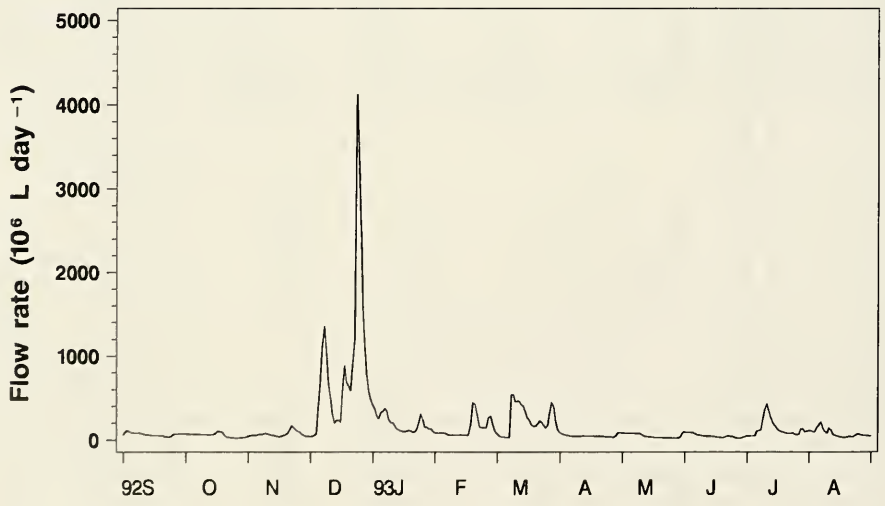

Figure 1. Flow rates over Penrith weir in the Hawkesbury-Nepean River.

Table 2. Simple correlation coefficients between environmental variables and mean cell density of autotrophic picoplankton in the Hawkesbury-Nepean River. All values except temperature were transformed by $\log_{10}$. n = 21-24 for each correlation coefficient. For overall correlation coefficients, n = 66-70. Significance levels: $0.01<p<0.05$*; $0.001<p<0.01$**; $p<0.001$***; NS = not significant.

Site	Flow	Temperature	Total phosphorus	Total nitrogen	Chlorophyll *a*
Penrith	0.14^{NS}	0.44*	0.22^{NS}	-0.03^{NS}	0.55**
North Richmond	-0.12^{NS}	0.80***	-0.18^{NS}	-0.67***	-0.01^{NS}
Sackville	-0.37^{NS}	-0.12^{NS}	-0.18^{NS}	-0.33^{NS}	0.40^{NS}
Overall	-0.07^{NS}	0.46***	0.04^{NS}	-0.06^{NS}	0.28*

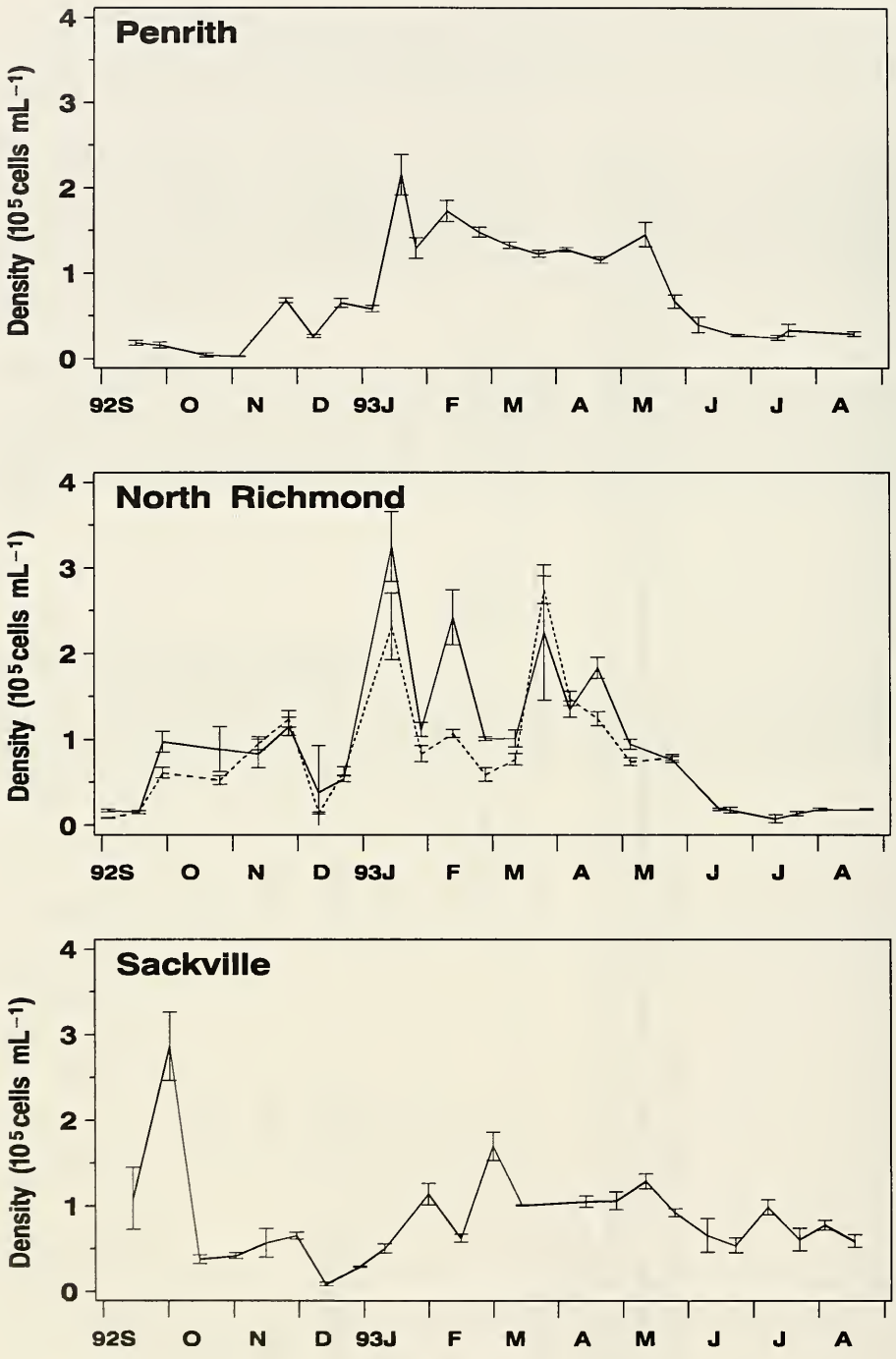

Figure 2. APP in the Hawkesbury-Nepean River: Solid lines = mean density ± SD at 1 m deep (n=4); dotted lines at North Richmond = mean density ± SD at 4 m deep (n=4).

APP at 1 m depth

APP were present at all three study sites throughout the year (Fig. 2). Cell density tended to be high between mid-summer and early autumn at Penrith and North Richmond. Over the sampling period, cell density varied two orders of magnitude from 2.2 x 10^3 to 3.2 x 10^5 cells ml^{-1}.

There were three morphologically distinctive cells: coccoid, ellipsoid and rod-shaped. In terms of proportional occurrences, coccoid cells became more important downstream from 25 to 52% of total cells during the study, whereas ellipsoid and rod-shaped cells decreased downstream from 64 to 47% and from 11 to 1% of total cells, respectively (Fig. 3)

Vertical Distribution of APP between 1 and 4 m depth

Between September 1992 and May 1993, the cell density of APP at 1m deep ranged from 1.5 x 10^4 to 3.2 x 10^5 cells ml^{-1} (overall mean 1.2 x 10^5 cells ml^{-1}) and the cell density of APP at 4 m deep ranged from 8.4 x 10^3 to 2.7 x10^5 cells ml^{-1} (overall mean 9.4 x 10^4 cells ml^{-1}) (Fig. 2). There was a significant positive correlation in cell densities of APP between depths ($r=0.94$, $n=18$, $p<0.0001$). Overall, the mean cell density of APP at 1 m deep was significantly different from that at 4 m deep ($p=0.0055$, $n=18$, paired-sample *t* test [two tailed] on $\log_{10}$-transformed cell density data).

Correlation between River Environmental Variables and APP Cell Densities

The pattern of associations between APP cell density and river environmental variables differed between sites (Table 2). The strongest correlation was found between APP cell density and temperature at North Richmond. Overall, the APP cell density was weakly but significantly positively correlated with temperature and total chlorophyll *a* (Table 2).

DISCUSSION

The present study is the first to report the presence of abundant APP, in the freshwater portion of a regulated coastal river in Australia and elsewhere. The range of recorded cell densities of APP in the Hawkesbury-Nepean is within the range reported for lakes of the Northern Hemisphere (Stockner 1991; Weisse 1993; Szelag-Wasielewska 1997) and is comparable to that in lakes of New Zealand (Burns and Stockner 1991). A change of two orders of magnitude in seasonal cell densities of APP at study sites also is within the range reported for temperate lakes (especially mesotrophic-eutrophic) where APP abundance may change seasonally by almost four orders of magnitude (Weisse 1993).

As has been reported for lakes (e.g. Fahnenstiel et al. 1991), the vertical heterogeneity of APP abundance exists in the Hawkesbury-Nepean River, at least at North Richmond. In lakes, higher densities of APP near the surface water are often observed during summer, coinciding with the development of summer thermal stratification (Stockner 1991; Gaedke and Weisse 1998). In the present study, the vertical heterogeneity of APP was examined only for limited duration at a single site. It is difficult to clearly demonstrate such a seasonal pattern in the vertical distribution of APP in the Hawkesbury-Nepean River. Nevertheless, the surface APP cell density tended to be higher than the deep APP cell density at North Richmond in January and February during the summer of 1992 and 1993 (Fig 2).

The three morphologically distinctive types of APP cells are present in different proportions in time and space in the Hawkesbury-Nepean River. A variety of morphological cell types of prokaryotic APP (including coccoid, ovoid and rod-shaped) has been reported for lakes, but there is currently no key available for the identification of proper APP species (MacIsaac and Stockner 1993; Weisse 1993). This is chiefly because the majority of APP "forms" are prokaryotes that lack cell organelles and internal structures useful for

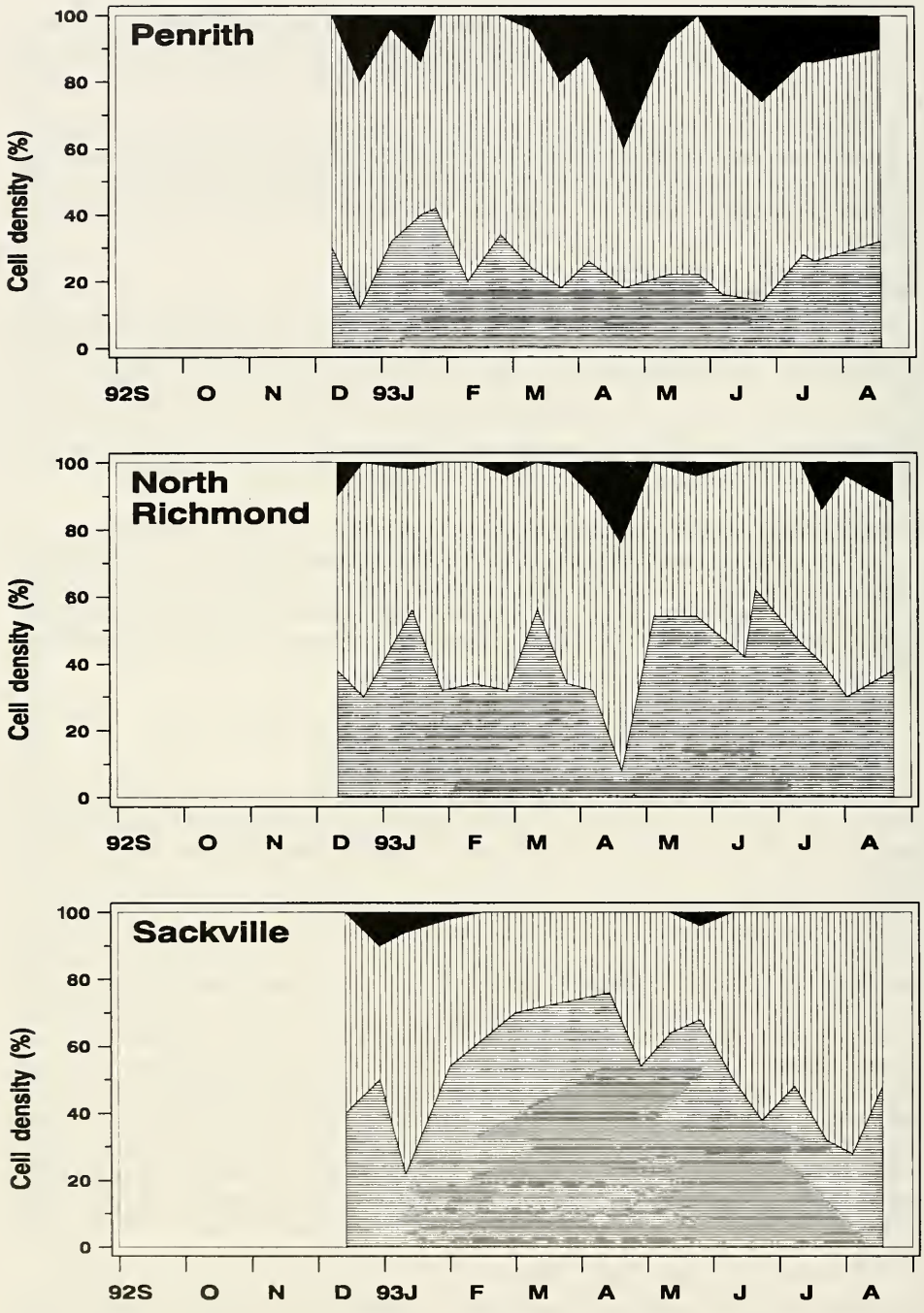

Figure 3. Relative cell densities (%) of morphologically distinctive APP in the Hawkesbury-Nepean River. Horizontal shading = coccoid cells; vertical shading = ellipsoid cells; black = rod-shaped cells.

the identification of species (Weisse 1993). Moreover, Weisse (1993) notes that even the morphologically uniform phycoerythrin-rich cyanobacteria may consist of more than one species. The taxonomic status of APP in the Hawkesbury-Nepean River awaits further studies.

In the present study, there was no strong, consistent pattern of temporal associations between river environmental variables and cell densities of APP among study sites. The positive correlation of APP cell density with temperature at Penrith and North Richmond is, nevertheless, in accord with similar findings for some of the temperate lakes (Kennaway and Edwards 1989; Burns and Stockner 1991). Nutrients and total phytoplankton biomass (as measured by total chlorophyll *a* concentrations) also showed a certain degree of correlation with APP cell densities. On the other hand, river flow, which is often strongly associated with the seasonal variation in density of river microplankton (Kobayashi et al. 1998 and reference therein) was not a significant correlate of APP at any of the three study sites. Overall, the relatively low correlation coefficients indicate that large variability is associated with the seasonal relationship between the examined environmental variables and APP cell densities in the Hawkesbury-Nepean River.

In an overview of APP from marine and fresh water ecosystems, Stockner (1988) has stressed the necessity of incorporating APP into conceptual models of lake plankton food webs, especially in the pelagic zone of ultra-oligotrophic systems. In these systems, APP may be key components in carbon metabolism and energy transfer, along with their heterotrophic counterparts (bacteria). Although this view is based on studies in freshwater lakes, APP may also need to be incorporated into a conceptual model of river plankton food webs.

As in other rivers, microzooplankton predominate in the Hawkesbury-Nepean (Kobayashi et al. 1998). Many species of microzooplankton have been reported to effectively consume picophytoplankton (Stocker and Antia 1986; Weisse 1988; Müller et al. 1991). Thus, APP may occupy an important trophic niche in plankton food webs of the river. In New Zealand lakes, Burns and Stockner (1991) have observed many picophytoplankton cells even in the gut of the small cladocerans such as *Ceriodaphnia dubia* and *Bosmina meridionalis*, although the digestion of the ingested cells by the cladocerans was not clearly demonstrated. *Bosmina meridionalis* occurs in the Hawkesbury-Nepean River and the microzooplankton community of the river consumes small algal food particles of ~5 μm in diameter (Kobayashi et al. 1996). Further studies of the ecology of APP are warranted, especially in relation to the trophic role of APP in the plankton food webs in the Hawkesbury-Nepean River.

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